Wilt and Root Diseases of *Asclepias tuberosa* L.

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ABSTRACT

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Chlorosis and wilting, followed by weak flowering or death, have been occurring in *Asclepias tuberosa* plants grown in Israel. Roots have been rotted and tuberous roots have shown dark lesions, sometimes with sclerotia. A binucleate *Rhizoctonia* sp. was detected on 39% and *Pythium intermedium* on 23% of diseased samples. Other organisms were less prevalent. Wilting began 7 days after seedlings were inoculated with *P. intermedium* or the binucleate *Rhizoctonia* sp. or both, with disease incidence ranging from 25 to 65% and disease severity index ranging from 0.30 to 0.85 (scale of 0 to 3). Healthy tuberous roots inoculated with the binucleate *Rhizoctonia* sp. exhibited dry rot within 4 to 7 days, and irregular black sclerotia of the pathogen were present 2 weeks later. This is the first report of either a binucleate *Rhizoctonia* sp. or *P. intermedium* occurring in *A. tuberosa*.

Asclepias tuberosa L., butterfly weed, is a perennial plant native to North America that has been grown in the United States mainly as an outdoor garden ornamental (9). Because of its attractive orange-red inflorescence, it has been developed recently as a cut-flower crop in Europe and Israel (7). The plant is propagated sexually by seeds (4) and vegetatively by tuberous root cuttings (2). It is self-incompatible for pollination and plants derived from seeds are genetically heterogeneous, particularly in growth habit and in flower color pattern (17). In Israel, the flowers are grown in heated greenhouses at 25/18°C (day/night) with a long-day photoperiod (12 to 14 h of natural daylight plus incandescent light during the night; 7). They are grown in sandy soil or in a soilless medium of volcanic stones and are drip-irrigated. Fumigation of the potting medium with methyl bromide (750 kg/ha) prior to planting is a common practice.

Recently, leaf chlorosis and wilting of plants, followed by weak flowering or plant death, has occurred in many commercial greenhouses. Typically, brown-to-black lesions are observed on the roots and tuberous roots of affected plants, sometimes progressing to a general necrosis characterized by a dry or soft rot (13). With moderate disease symptoms, emer-

gence of young buds is retarded and the numbers of stems and flowers are reduced. When disease is severe, new buds do not develop. Both the yield and the quality of flowers are affected. Disease incidence and severity are dramatically increased after harvesting flowers by cutting flower stems at their base. The objective of the study was to identify the pathogens associated with these disease symptoms on *A. tuberosa*.

MATERIALS AND METHODS

Isolation and identification of pathogens from diseased plants and seeds. Between October and February 1994, random samples of diseased plants from 26 greenhouses, 5 to 7 plants in each, were examined in the laboratory. Plants were between 3 and 15 months old, depending on the planting date. Five-millimeter segments from roots, tuberous roots, root crowns, and stems of diseased plants were washed, surface-sterilized with 0.3% HClO, and placed on different growth media. The isolation media were: (i) potato dextrose agar (PDA, Difco Laboratories, Detroit) supplemented with streptomycin sulfate, 100 ppm; (ii) sorbose (SA), 2 g/liter; agar, 18 g/liter; streptomycin sulfate, 100 ppm; and (iii) cornmeal agar (CMA; Difco Laboratories).

When soft rot was present, tuberous roots were ground in saline and plated on crystal violet pectin medium (CVP; 6) to detect the presence of *Erwinia* spp. Plates were incubated at 27 and 33.5°C for 48 h, and bacteria were identified on the basis of cavity formation pattern of colonies (11).

Five hundred seeds were randomly sampled from each of 25 seed lots, according

to the Israeli standard of testing seedborne pathogens in tomato seeds (500 of 100,000 seeds [500 g]). All seed lots arriving in Israel from the United States, Japan, Holland, or Germany, and those that were self-propagated in Israel, 16 lots in 1994 and 9 in 1995, were checked. Seeds were washed, surface-sterilized, and placed on PDA and CMA. Pathogens were identified after incubation of samples on growth media for several days at 27°C.

Inoculation of plants and tuberous roots. Two isolates of a binucleate *Rhizoctonia* sp., one originating from infected seeds (Rs) and the other from tuberous roots (Rr), and one isolate of *Pythium intermedium* from tuberous roots, were used for inoculations. To prepare the inoculum, agar containing the fungal mycelium grown on PDA for 3 days at 27°C was blended for 30 to 60 sec with sterile water. Concentrations of inoculum, examined by serial dilution and plating on PDA, were 2×10^6 , 3×10^5 , and 5×10^5 CFU/ml for Rs, Rr, and *P. intermedium* isolates, respectively.

Seedlings for inoculation were grown from seeds disinfected with hypochlorite (0.3% for 3 min), in trays containing a sterile vermiculite/sand mixture (1:3) for 3 to 4 weeks (until emergence of two true leaves). Seedlings were inoculated by dipping their roots in the fungal mixture for 20 min. Control plants were dipped in water. Plants were grown in a growth chamber at 22°C, with 10 h of illumination at a light intensity of $100 \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The experimental design was a completely randomized block design, with five replications, and four seedlings in each experimental unit. For each treatment, $n = 20$. The experiment was repeated three times.

Wilting and damping-off symptoms were evaluated on a scale of 0 to 3, where 0 = healthy plant, 1 = moderate wilt, 2 = severe wilt, and 3 = dead plant (damping-off). Fungi were reisolated from seedlings 35 days after inoculation by placing 5-mm-long segments of roots or stem bases on growth media, as described previously.

Healthy tuberous roots (3 to 4 cm diam) for inoculation were surface-sterilized with 0.3% HClO, and the tissue was punctured to a depth of 2 to 3 mm with a sterile scalpel. An agar disc, 1 cm in diameter, containing *P. intermedium* or Rr, was placed on the wounded tissue. The roots were incubated on wet paper towels in a box at

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Fig. 1. Incidence of pathogens isolated from diseased plants in commercial greenhouses. Diseased plants were arbitrarily selected from 26 greenhouses, 5 to 7 plants from each greenhouse: binucleate *Rhizoctonia* sp. (Rhiz); *Pythium intermedium* (Pyt); binucleate *Rhizoctonia* sp. + *P. intermedium* (R+P); *Fusarium oxysporum* (F. oxy); and *Erwinia carotovora* subsp. *carotovora* (Erw).

Table 1. Incidence of a binucleate *Rhizoctonia* sp. and *Pythium intermedium* in *Asclepias tuberosa* seed lots in 1994 and 1995^a

Pathogen	Infected lots (%)		Level of infection within a lot	
	1994	1995	1994	1995
Binucleate <i>Rhizoctonia</i> sp.	18.8	22.2	4.5	1.3
<i>P. intermedium</i>	0	0	0	0

^a Sixteen seed lots were examined in 1994 and nine in 1995. From each lot, 500 seeds were randomly sampled and tested for pathogens.

Table 2. Percent disease incidence for seedlings inoculated with selected pathogens

Treatment ^a	Days after inoculation		
	7	22	35
<i>Pythium intermedium</i>	50 ab ^c	85 a	85 a
Binucleate <i>Rhizoctonia</i> (Rr)	25 bc	40 b	75 a
Binucleate <i>Rhizoctonia</i> (Rs)	35 ab	65 ab	90 a
<i>P. intermedium</i> & binucleate <i>Rhizoctonia</i> (Rr)	65 a	65 ab	75 a
Control	0 c	20 c	45 b

^a Plants were inoculated by dipping roots in a mycelial suspension at concentrations 2×10^6 , 3×10^5 , and 5×10^5 CFU/ml for Rs, Rr, and *P. intermedium* isolates, respectively. Control plants were dipped in water.

^c Means followed by the same letter within a column are not significantly different ($P < 0.05$).

27°C and 95% RH for 7 to 14 days. Roots were evaluated for presence or absence of rot or sclerotia.

Statistics. Disease incidence and severity were analyzed by analysis of variance. Means were compared with Duncan's multiple range test at a significance level of $P < 0.05$. Percentages were arcsine-transformed before analysis.

RESULTS

Isolation and identification of pathogens from diseased plants. Symptoms in plants from commercial greenhouses included chlorosis of leaves and general wilting of the plant. Roots were either rotted or had brown lesions. On tuberous roots, mainly on the upper portion, brown to black lesions were observed, and sometimes black, irregularly-shaped sclerotia were present.

The most prevalent pathogens isolated from plants that were chlorotic and wilted were a binucleate *Rhizoctonia* sp. (39% of samples), *P. intermedium* (23% of samples), or a combination of the binucleate *Rhizoctonia* and *P. intermedium* (15.4% of the samples) (Fig. 1). The binucleate *Rhizoctonia* sp. was isolated primarily from tuberous roots, and *P. intermedium* was isolated primarily from the stem base.

The nuclear status of all *Rhizoctonia* isolates stained with HCl-Geimsa (12) was binucleate, with appearance resembling AG-Ba or AG-Bb. Identification of anastomosis group was carried out on water agar (8), but the isolates did not anastomose with any of the following binucleate testers: AG-A, AG-Bb, AG-D, AG-E, AG-F, AG-G, AG-I, AG-K, AG-P, AG-Q, AG-R, AG-S. *P. intermedium* was identified by terminal or intercalary spherical catenulate

hyphal swellings formed on agar (15).

Fusarium oxysporum was isolated in 7.7% of the samples. When lesions on the tuberous roots were accompanied by soft rot, *Erwinia carotovora* subsp. *carotovora* was usually isolated (7.7%, Fig. 1). A *Myrothecium* sp. was isolated in a few cases, mostly from plants grown in soilless media (perlite or volcanic stone particles). Pathogenicity studies were not conducted on these organisms because of their relatively low frequency of isolation.

Detection of pathogens on *A. tuberosa* seeds. Of 25 seed lots sampled from different sources, 5 were found to be infected with the binucleate *Rhizoctonia* sp. (3 in 1994 and 2 in 1995). The average rates of infection were 4.5% in 1994 and 1.3% in 1995 (Table 1). Germinating seeds on which the binucleate *Rhizoctonia* sp. was detected died within a few days. *Pythium* spp. were not detected in any of the lots. Several *Fusarium* species at various levels (0.1 to 8%) were observed in 10 lots; however, *F. oxysporum* or *F. solani* were not detected in any of them, although they had been isolated from several of the dead plants. Other saprophytic fungi observed included *Alternaria alternata*.

Inoculation of seedlings and tuberous roots. *A. tuberosa* seedlings inoculated with Rr, Rs, *P. intermedium*, or a combination of Rr and *P. intermedium*, showed symptoms of wilting after 7 days (Table 2). Numerically, 7 days after inoculation, a mixture of Rr and *P. intermedium* caused a higher disease incidence (65%) and symptom severity index (0.85 on a scale of 0 to 3) than plants inoculated with either of the pathogens alone (Tables 2 and 3), although differences were not statistically different. However, 22 and 35 days after inoculation, seedlings inoculated with a single pathogen or with combined pathogens did not differ in their disease incidence or severity index, but had greater incidence and severity than the control. Differences between the Rs and Rr isolates may be attributable to the fact that the concentration of Rs was almost 10-fold higher than that of Rr. The pathogens used for inoculation were reisolated from roots and root crowns of the seedlings: 100% recovery for binucleate *Rhizoctonia* from Rr and Rs inoculated plants; 93% recovery for *P. intermedium* from plants inoculated with this isolate; 90 and 70% recovery for binucleate *Rhizoctonia* sp. and *P. intermedium*, respectively, from plants inoculated with both pathogens. Seedlings in control pots had low disease incidence and only *Fusarium* spp. were isolated.

Healthy tuberous roots inoculated with Rr showed symptoms of dry rot within 4 to 7 days; by 2 weeks after inoculation, typical black sclerotia were observed. Inoculation with *P. intermedium* resulted in a limited pale-brown rot that was localized around the wound and did not develop further.

Table 3. Disease severity index^y in seedlings inoculated with selected pathogens

Treatment ^z	Days after inoculation		
	7	22	35
<i>Pythium intermedium</i>	0.60 ab	2.00 a	2.30 a
Binucleate <i>Rhizoctonia</i> (Rr)	0.30 ab	1.10 ab	1.70 b
Binucleate <i>Rhizoctonia</i> (Rs)	0.70 a	1.80 a	2.30 a
<i>P. intermedium</i> & binucleate <i>Rhizoctonia</i> (Rr)	0.85 a	1.75 a	2.00 ab
Control	0.05 b	0.50 c	0.80 c

^y Disease severity was evaluated on a scale of 0 to 3, where 0 = healthy plant, 1 = moderate wilt, 2 = severe wilt, 3 = dead plant (damping-off). Means followed by the same letter within a column are not significantly different ($P < 0.05$).

^z Plants were inoculated by dipping roots in a mycelial suspension at concentrations of 2×10^6 , 3×10^5 , and 5×10^5 CFU/ml for Rs, Rr, and *P. intermedium* isolates, respectively. Control plants were dipped in water.

DISCUSSION

Leaf chlorosis, weak flowering, wilting of plants, and rotting of tuberous roots in *A. tuberosa* plants grown in commercial greenhouses in Israel are predominantly caused by two pathogens, a binucleate *Rhizoctonia* sp. and *P. intermedium*. The findings of this study indicate a high probability that the material used for propagation, whether seeds or tuberous roots, may be responsible for the dissemination of these pathogens in *A. tuberosa*. *R. solani* is known to be disseminated by seeds of different crops; for example, amaranthus (1), pea (18), and cotton (10). It is also disseminated by potato tubers (14,16).

Pathogenicity of these organisms to *A. tuberosa* when inoculated either alone or in combination was demonstrated. Inoculation with a combination of both pathogens was not characterized by a synergistic interaction. This is the first report of either a binucleate *Rhizoctonia* sp. or *P. intermedium* occurring in *A. tuberosa*, although *R. solani* has been reported in other species of *Asclepias* (3).

Disease incidence in commercial greenhouses varied between 20 and 50%, depending on growth conditions: crop age (whether it was first, second, or third year), growth medium (sandy soil, or soilless medium such as perlite or volcanic stone particles), irrigation frequency (long intervals between irrigation, or constant water

content), and propagative material (seeds or tuberous roots). In greenhouses in which seeds were used, pathological problems started only after several cycles of flower harvesting; where tuberous roots were used, diseased plants were detected soon after planting, apparently due to latent infections. Fumigation of the soil or soilless medium with methyl bromide, a routine procedure in the management of this crop, might increase the development and expression of the disease if the propagative material is latently infected (5). As a consequence of this study, growers now treat their crops with fungicides specific for *Rhizoctonia* spp. and *Pythium* spp. to reduce economic damage.

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